

Relative GC-MS Examination of Biological Activity Constituents of *Ocimum tenuiflorum* Extracts

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RESEARCH ARTICLE

Relative GC-MS Examination of Biological Activity Constituents of *Ocimum tenuiflorum* Extracts

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ABSTRACT:

Plants from the genus *Ocimum* belonging to the family *Lamiaceae* (Ordo Lamiales), also known as tulsi, are widely distributed in tropical, subtropical, and warm climates throughout the world, and are among the types of medicinally effective herbal plants. Tulsi is referred to as the "Herbal Queen" because of its reputation for producing aromatic oils. In India, this plant is primarily grown at home for religious purposes and temple offerings. In traditional Indian medicine, plants of the genus *Ocimum* are widely employed. The tulsi plant is not commonly used as an alternative to herbal medicine in Bali, and there is a dearth of literature describing its chemical constituents and secondary metabolites. In addition, the secondary metabolites of tulsi plants growing in India and Bali are influenced by the distinct geographical conditions of their respective growing environments. This study intends to evaluate the secondary metabolite chemicals and biological aspects of Bali-grown tulsi plants. Synthesis of simplicia, followed by maceration, evaporation, and GC-MS analysis, is used to evaluate the chemical structure of secondary metabolites in tulsi extract. In this study, ethanol (polar) and chloroform (semi-polar) were employed to isolate secondary metabolites with varying degrees of polarity. Chloroform solvent successfully isolated secondary metabolites at high concentrations, including Eugenol, Copaene, Cyclohexane, Caryophyllene, Humulene, Germacrene D, Naphthalene, Caryophyllene oxide, Phthalic acid, 9,12,15-Octadecatrienoic acid, Dibutyl phthalate, and Caryophyllene oxide (linolenic acid). While the ethanol extract could only isolate Eugenol, Alpha-Copaene, Cyclohexane, Caryophyllene, Germacrene D, and N-Desmethylpentol. The potential biological effects as natural antibacterial and antifungal agents of the identified compounds in both extracts are highlighted. Our findings support the use of both extracts to treat comparable medical conditions, including bacterial and fungal infections, as supported by empirical evidence. Due to their antiseptic, analgesic, anti-inflammatory, antibacterial, immunomodulatory, hypoglycemic, hypotensive, cardioprotective, and antioxidant properties, numerous secondary metabolites in these two forms of tulsi extract have the potential to be developed as therapeutic agents.

KEYWORDS: Tulsi, GC-MS, secondary metabolites.

INTRODUCTION:

Phytoconstituents (phyto-' meaning 'plant' in Greek) or phytochemicals are bioactive ingredients found in numerous plant species.^{1,2} Naturally, these chemicals play a crucial function in protecting plants from microbial or insect-caused infection or attack.^{3,4}

The production of medicinal plants based on ethnomedicine for a variety of diseases, such as tuberculosis, cancer, and inflammatory disorders such as diabetes, is a popular community preference.^{5,6} If handled correctly, the usage of medicinal herbs is often safer in terms of side effects and toxicity compared to contemporary pharmaceuticals.^{2,7,8} The chemical compositions of plants utilized in phytomedicine are complicated and comprise numerous components. The age of the plant, geographical origin (climatic differences), growing conditions, genetic composition of plants, varieties, plant species, and portions of plants

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chosen for processing all influence the quality of the elements and the content of the active components in herbal medicines.^{9,10,11}

The plants of the genus *Ocimum*, which are members of the family Lamiaceae (Ordo Lamiales) and are known as tulusi, are extensively distributed in tropical, subtropical, and warm climates all over the world.^{12,13} It is classified as types of herbal plants that have been demonstrated to have medicinal benefits.^{14,15} This plant is reported to produce an oil containing a variety of aromatic chemicals. Secondary metabolites (essential oils) of the genus *Ocimum* are recommended for the treatment of malaria, bronchitis, diarrhea, dysentery, etc. due to their anticancer, antioxidant, antifungal, and anti-inflammatory effects.^{16,17} *Ocimum* species have been found to contain significant metabolites such as linalool, linalyl, geraniol, citral, camphor, eugenol, methyleugenol, methyl chavicol, methyl cinnamate, thymol, safrole, taxol, urosolic acid, etc. In the pharmaceutical, perfume, and cosmetics industries, these metabolites are highly lucrative.^{15,18,19}



Figure 1. a. Khrisna Tulsi, b. Rama tulsi

As in India, various types of Tulsi plants grow in Indonesia, including on the island of Bali, with Tulsi Rama and Tulsi Krishna being the most prevalent and widely utilized as herbal medicine or tea. The stems, leaves, and flowers of the two types of tulsi are distinct in terms of color. These morphological differences may result in distinct secondary metabolite compositions. Consequently, the profile of secondary metabolites from Tulsi Rama was characterized in this study, and future research will do the same for Tulsi Krishna.

In India, plants of the genus *Ocimum* are commonly utilized in traditional medicine, however in Bali, tulsi is not frequently used as an alternative to herbal medicine, and there is a dearth of research describing its chemical constituents and secondary metabolites. Geographical and genetic variables of the plant itself influence the content of plant metabolites, allowing for variances in metabolites and the associated pharmacological

reactions.¹⁵ Therefore, it is necessary to standardize the extract as a raw material for the phytopharmaceutical in order to be accounted for medically. In this study, polar 95% ethanol and non-polar chloroform solvents were used in the maceration procedure. The purpose of using these two solvents is to characterize all types of secondary metabolites in the tulsi plant, polar and non-polar alike.

MATERIALS AND METHODS:

Sample Collection and Extraction:

The sample utilized was collected at an altitude of 425 masl in the city of Gianyar. The organs used as samples were stems and leaves. The samples were dried in an oven at a temperature between 50 and 60 degrees Celsius to achieve a constant dry mass. To be able to characterize all sorts of secondary metabolites with differing polarities, two solvents with varying polarity levels are utilized. The sample was pulverized using a blender and macerated with 95% ethanol and chloroform at a ratio of 3:1 (sample powder: ethanol/chloroform) in a sealed aluminum foil container (vacuum sealed aluminum). Each 24 hours of the 46-hour maceration process were filtered, and the sample was soaked in new 95% ethanol and chloroform. The extract was then filtered using filter paper to obtain the filtrate, which was then evaporated in the dark using an evaporator to produce a precipitate. The evaporation of the precipitate results in the production of crude extract (ethanolic and chloroform extract).

Secondary Metabolites Characteristics (GC-MS test):

In addition, the ethanol and chloroform extracts will be phytochemically evaluated with GC-MS Agilent 7890B and MSD 5977 A. The GC-MS was injected with a 1 μ L sample.

RESULTS:

In the ethanolic extract of tulsi, six types of secondary metabolites with GC-MS peak quality above 60% were found. With a percentage of covered area of 44.12%, Cyclohexane,1-ethenyl-1-methyl-2,4-bis(1-methylethyl) had the highest concentration, followed by Eugenol (17.20%), Germacrene D (10.24%), Caryophyllene (10.23%), N-Desmethyl tapentadol (3.56%), and Alpha-Copaene (1.92%). In addition, retention time, molecular formula, molecular weight, and boiling point information for each of these secondary metabolites is presented in table 1. Figure 1 depicts the GC-MS chromatogram of the ethanolic extract of tulsi (*Ocimum tenuiflorum*).

Table 1: Chemical constituents (secondary metabolites) found in tulsi ethanolic extract (*Ocimum tenuiflorum*)

Constituents	Type of secondary metabolites	Retention Time (RT) (Minutes)	Molecular formula	Molecular Weight (g/mol)	Peak area	Boiling point
Eugenol (4-allyl-2-methoxyphenol)	Terpenoid-alkaloids	7.153	C ₁₀ H ₁₂ O ₂	164.2	17.20	254°C
Alfa-Copaene	Sesquiterpenes	7.335	C ₁₅ H ₂₄	204.35	1.96	248.5°C
Cyclohexane, 1-ethenyl-1-methyl-2,4-bis(1-methylethenyl)	Benzene	7.426	C ₁₅ H ₂₄	204.35	44.12	80.75°C
Caryophyllene	Sesquiterpenes	7.665	C ₁₅ H ₂₄	204.36	10.23	245.3°C
N-Desmethyl tapentadol	Alkylbenzene	9.038	C ₁₃ H ₂₁ NO	207.31	3.56	123°C
Germacrene D	Sesquiterpenes	8.112	C ₁₅ H ₂₄	204.35	10.24	236.4°C

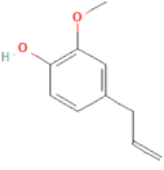
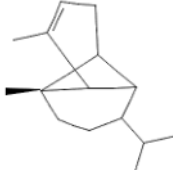
Table 2: Chemical constituents (secondary metabolites) discovered in chloroform tulsi extract (*Ocimum tenuiflorum*)

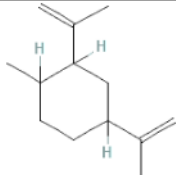
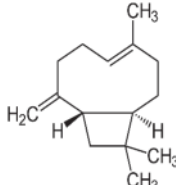
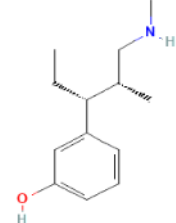
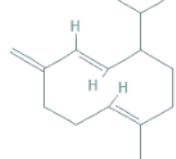
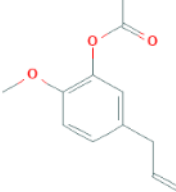
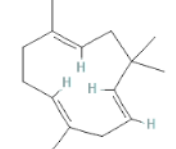
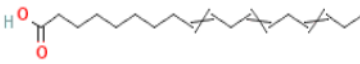
Constituents	Type of secondary metabolites	Retention Time (RT) (Minutes)	Molecular formula	Molecular Weight (g/mol)	Peak area	Boiling point
3-allyl-6-methoxyphenol	Phenolic	7.152	C ₁₂ H ₁₆ O ₃	206.24	27.82	127°C
Cyclohexane, 1-ethenyl-1-methyl-2,4-bis(1-methylethenyl)	Benzene	7.379	C ₁₅ H ₂₄	204.35	30.81	80.75°C
Copaene	Sesquiterpenes	7.335	C ₁₅ H ₂₄	204.35	1.42	248.5°C
Caryophyllene	Sesquiterpenes	7.665	C ₁₅ H ₂₄	204.36	6.55	245.3°C
Humulene	Sesquiterpenes	7.911	C ₁₅ H ₂₄	204.35	0.56	106°C
Germacrene D	Sesquiterpenes	8.112	C ₁₅ H ₂₄	204.35	7.94	236.4°C
Naphthalene	Aromatic compounds	8.404	C ₁₀ H ₈	128.1705	1.25	218 °C
Caryophyllene oxide	Sesquiterpenes	9.038	C ₁₅ H ₂₄ O	220.3505	2.61	279.6°C
9,12,15-Octadecatrienoic acid	Methylester, Polyunsaturated fatty acid	16.698	C ₁₈ H ₃₀ O ₂	278.43	4.48	443.4°C
N-Desmethyl tapentadol	Alkylbenzene	8.704	C ₁₃ H ₂₁ NO	207.31	0.50	123°C
Dibutyl phthalate	phthalate ester	13.243	C ₁₈ H ₂₆ O ₄	278.3	4.30	340°C

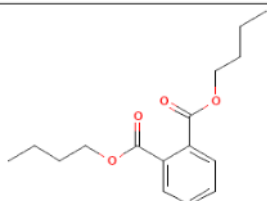
Similar to the ethanol extract of the tulsi plant, the chloroform extract of the tulsi plant contained numerous types of chemicals belonging to the secondary metabolites of the phenolic, benzene, sesquiterpenes, aromatic, alkylbenzene, and two types of ester compounds that were not present in the ethanol extract of the tulsi plant. In this chloroform extract, more secondary metabolites (12 types) were extracted than in the ethanol extract of tulsi (6 types), with GC-MS peak quality exceeding 60%. Most of the secondary

metabolites extracted with ethanol were also present in the chloroform extract, but in lower amounts. These included Cyclohexane, 1-ethenyl-1-methyl-2,4-bis(1-methylethyl), Germacrene D, Caryophyllene, N-Desmethyl tapentadol, and Copaene. The chloroform extract of the tulsi plant contained lower levels of all secondary metabolites than the ethanol extract. Eugenol (4-allyl-2-methoxyphenol) was discovered only in the ethanol extract, but not in the chloroform extract.

Table 3: Advantages of secondary metabolites found in ethanol and chloroform extract of tulsi (*Ocimum tenuiflorum*)

Constituents	Chemical Structure	Biological potential
Eugenol (4-allyl-2-methoxyphenol)		Biological activity as an antifungal, antibacterial, antimicrobial, anti-inflammatory, analgesic, antioxidant, anticancer, and antiparasitic agent. ²⁰⁻²²
Alfa-Copaene		Antibacterial efficacy against several <i>Streptococcus</i> bacterium, <i>Actinomyces naeslundii</i> , <i>Bacteroides fragilis</i> , <i>Prevotella nigrescens</i> ²³ and <i>Xanthomonas oryzae</i> ²⁴

Cyclohexane, 1-ethenyl-1-methyl-2,4-bis(1-methylethenyl)		Strong scent, flavor, and spice level. Additionally, it has been demonstrated to suppress various types of harmful microorganisms. ^{25,21}
Caryophyllene		Numerous in vitro and in vivo investigations demonstrate that caryophyllene has an anti-inflammatory activity. ⁷ In addition, it has anticonvulsant, analgesic, myorelaxing, sedative, and antidepressant properties. ^{8-10.}
N-Desmethyl tapentadol		possesses qualities suitable for development as an analgesic medication ^{26,27}
Germacrene D		It exhibits antiparasitic, anti-inflammatory, antibacterial, and anti-worm activities and has pesticide potential. ^{28,29}
3-allyl-6-methoxyphenol		Contains antibacterial, antifungal, and antioxidant qualities, as well as pesticide potential. ^{30,31}
Humulene		In addition to possessing antioxidant, anti-inflammatory, and antibacterial characteristics, it has the potential to be developed as a larvicide. ³²⁻³⁴
9,12,15-Octadecatrienoic acid		It has been shown to lessen the risk of fatal ischemia in patients with heart disease due to its antioxidant and anti-inflammatory characteristics. Additionally, it possesses anticancer properties. ^{35,36}

Dibutyl phthalate		<p>In addition to their antibacterial and antifungal characteristics, these chemicals also possess hazardous qualities, particularly with regard to reproduction.³⁷</p>
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Other secondary metabolites exclusive to chloroform extract were 3-allyl-6-methoxyphenol (27.82%), Humulene (0.56%), Naphthalene (1.25%), Caryophyllene oxide (2.61%), 9,12,15-Octadecatrienoic acid (4.48%), and Dibutyl phthalate (4.30%). According to the lists of secondary metabolites in tables 1 (ethanol extract) and 2 (chloroform extract), the secondary metabolites recovered by the chloroform solvent were more diverse than those isolated by the ethanol solvent. Although Cyclohexane, 1-ethenyl-1-methyl-2,4-bis(1-methylethyl) was the most abundant secondary metabolite in the ethanol and chloroform extracts, its concentration was higher in the ethanol extract than in the tulsi chloroform extract. In addition, the chloroform extract contained lower amounts of additional chemicals

than the tulsi ethanol extract. Eugenol is the sole type of secondary metabolite molecule detected in tulsi ethanol extract (4-allyl-2-methoxyphenol). In addition, the retention time, molecular formula, molecular weight, and boiling point for each of these secondary metabolites are detailed in Table 1 and 2. Figure 1 and 2 represents the GC-MS chromatogram of the ethanolic and chloroform extract of tulsi (*Ocimum tenuiflorum*). The chemical structure of each secondary metabolite component discovered in the ethanol and chloroform extracts of the tulsi plant, as well as the biological potential of those chemicals, are detailed in Table 3. Based on prior research, the majority of identified secondary metabolites have antibacterial and antifungal activities.

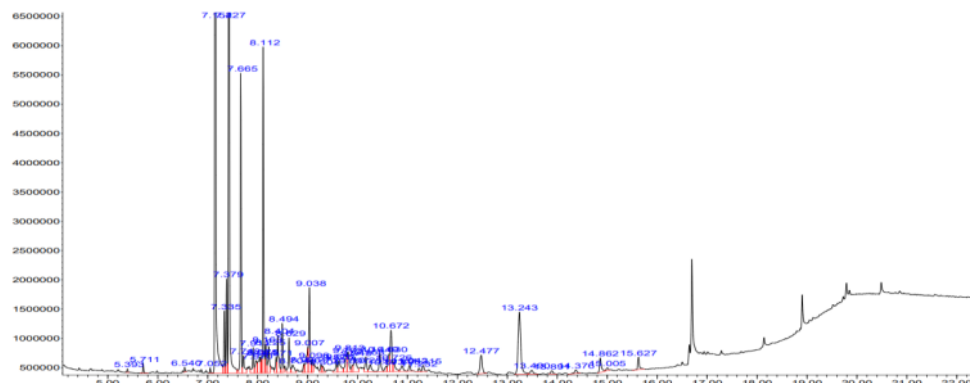


Figure 2. Chromatogram of GC-MS for ethanolic tulsi extract (*Ocimum tenuiflorum*)

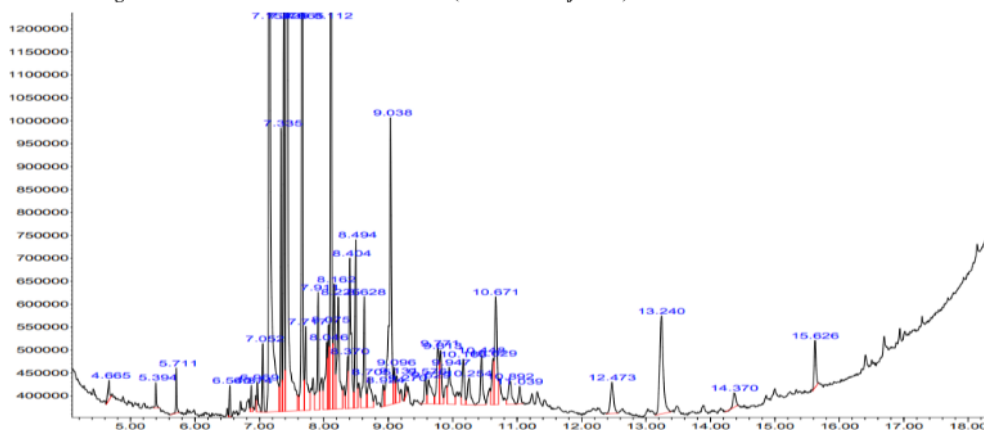


Figure 3. Chromatogram of GC-MS for chloroform tulsi extract (*Ocimum tenuiflorum*)

DISCUSSION:

In this investigation, two polarity-distinct types of organic solvents were utilized. In order to bind more polar/semipolar molecules, 95 percent ethanol is employed in the maceration process. While chloroform is used to bind the lipophilic group of secondary metabolites, chloroform is utilized to bind the hydrophilic group of secondary metabolites. Using either ethanol or chloroform as solvents, many types of secondary metabolites were discovered in tulsi extract.

Ocimum tenuiflorum tulsi extract contains antistress, antiseptic, analgesic, anti-inflammatory, antimicrobial, immunomodulatory, hypoglycemic, hypotensive, cardioprotective, and antioxidant activities, according to various research.^{9,38,39} The secondary metabolites discovered in the ethanol and chloroform extracts of the tulsi plant have diverse biological activities, as detailed in Table 3. Eugenol (4-allyl-2-methoxyphenol) is one of the secondary metabolites that has the potential to be developed as a therapeutic agent due to its antiseptic, analgesic, anti-inflammatory, antibacterial, immunomodulatory, hypoglycemic, hypotensive, cardioprotective, and antioxidant properties.²⁰⁻²²

In a number of investigations, cyclohexane and its derivative 1-ethenyl-1-methyl-2,4-bis(1-methylethyl) inhibited the development of harmful bacteria, including *S. aureus*, *S. aeruginosa*, and *E. coli*.²¹ Moreover, this secondary metabolite was found in significant concentrations in both the ethanol and chloroform extracts of the tulsi plant. It demonstrates that both types of extracts have a tremendous amount of potential as natural antibacterials derived from herbal plants. In addition, the Caryophyllene chemical, which is included in the Sesquiterpenes compound group, has been demonstrated in vitro and in vivo to have anti-inflammatory properties by various research.⁴⁰⁻⁴³ This substance not only has anti-inflammatory properties, but it can also impede the growth of *Streptococcus* bacteria.³⁴ This secondary metabolite molecule exhibits antidepressant properties; hence it has the potential to be developed as a sedative ingredient.²⁸

Moreover, a number of the compounds discovered in these two types of tulsi plant extracts have intriguing biological features, including the potential to be developed as larvicides and natural insecticides, especially against the *Aedes* mosquito, which is a vector for a number of dangerous infections.²⁸ Multiple studies of the Germacrene D, 3-allyl-6-methoxyphenol, and Humulene groups have revealed that, although being toxic to *Aedes* larvae and adult mosquitoes, these three compounds are not dangerous to humans at particular concentrations (proven by in vitro and in vivo tests). Consequently, the extract of the tulsi plant has the potential to be developed as both a larvicide and a

natural insecticide, despite the fact that multiple studies have proven that mosquitoes possess a resistance mechanism to chemical insecticides.²³ Consequently, the extract of the tulsi plant has tremendous potential to be developed as a larvicide as well as a natural insecticide, despite the fact that numerous studies have demonstrated that mosquitoes exhibit a resistance mechanism to chemical insecticides.¹⁹⁻²²

CONCLUSION:

Two different solvents, ethanol and chloroform, were used to extract secondary metabolites from the tulsi plant. The majority of these secondary metabolites exhibit antipathogenic properties, including antifungal and antibacterial activities. The cyclohexane molecule with the highest concentration in both ethanol extract and chloroform possesses antibacterial properties and has the potential to be developed into various antipathogens, such as antifungals. In addition, the Caryophyllene component, which belongs to the Sesquiterpenes compound family, has been demonstrated by multiple studies to have anti-inflammatory properties. Various secondary metabolites in these two forms of tulsi extract have the potential to be developed as therapeutic agents due to their antiseptic, analgesic, anti-inflammatory, antibacterial, immunomodulatory, hypoglycemic, hypotensive, cardioprotective, and antioxidant activities. There are variations in the types and amounts of secondary metabolites present in each extract, resulting in distinct biological effects/capabilities that require additional study.

CONFLICT OF INTEREST:

The authors have no conflicts of interest regarding this investigation.

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